PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 35/12, 37/02, 39/00
A61K 49/00, C07K 7/10, 13/00
C12Q 1/02, G01N 33/543, 33/564

A1

(11) International Publication Number: WO 90/10449

(43) International Publication Date: 20 September 1990 (20.09.90)

(21) International Application Number: PCT/US90/01397

(22) International Filing Date: 14 March 1990 (14.03.90)

(30) Priority data:

322,864	14 March 1989 (14.03.89)	US
371,249	26 June 1989 (26.06.89)	US

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), SU.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(71)(72) Applicants and Inventors: COHEN, Irun, R. [US/IL]; 11 Hankin Street, 76 100 Rehovot (IL). ELIAS, Dana [IL/IL]; Derech Yavne 57/13, 76 100 Rehovot (IL). MARKOVITS, Doron [IL/IL]; 11 Horovitz Street, 70 700 Gedera (IL).

(74) Agent: BROWDY AND NEIMARK; 419 Seventh Street, N.W., Washington, DC 20004 (US).

(54) Title: DIAGNOSIS AND TREATMENT OF INSULIN DEPENDENT DIABETES MELLITUS

(57) Abstract

A 65 KD heat shock protein, proteins cross-reactive therewith, antibodies thereto or T cells specific thereto can be used for detecting in humans the existence of, a tendency to develop, or the initiation of a process leading to insulin dependent diabetes mellitus. Antibodies to hsp65 can be used to detect the hsp65 molecule in blood or urine. The hsp65 molecule of any species, or any other substance immunologically cross-reactive therewith, when administered with a tolerogenic carrier, can be used for the prevention or treatment of IDDM prior to development of clinical symptoms thereof. T cells, active fragments thereof or the receptor peptide thereof can also be used for prevention or treatment of IDDM.

DIAGNOSIS AND TREATMENT OF INSULIN DEPENDENT DIABETES MELLITUS

Field of the Invention

The present invention relates to a method for detecting the existence of, a tendency to develop, or the initiation of a process leading to insulin dependent diabetes mellitus (IDDM), and, more particularly, to such a method which detects the presence of a 65 KD heat shock protein (hsp65) (or a molecule immunologically cross-reactive therewith) or antibodies or T cells reactive with such a protein.

The present invention further relates to a method for the prevention of IDDM or the treatment of IDDM in its incipient stages by administering hsp65 or an immunologically related protein or fragment, or by administering T cells activated by such protein or fragment, such T cells which have been treated to attenuate them or

immunogenicity, or fragments of such T cells or treated T cells, in such a manner as to cause immunological tolerance therefor.

Background of the Invention

The incidence of insulin dependent diabetes mellitus (IDDM) has risen several fold during recent decades in many countries and it is estimated that 1% of the people alive today will have developed IDDM before they reach the age of 70. IDDM is caused by an autoimmune process which destroys the insulin-producing beta cells. Diabetes becomes clinically evident only after the vast majority of beta cells are irrevocably destroyed (perhaps 90%) and the life of the individual becomes dependent on an exogenous supply of insulin. In other words, at the time of clinical diagnosis, the autoimmune process has already done irreversible damage, most of it without noticeable symptoms.

Successful treatment of the autoimmune process responsible for the disease ideally should be initiated before the patient has overt symptoms of diabetes and requires insulin replacement for his or her own lost capability to produce insulin. Termination of the autoimmune process would result in cure of the disease and prevention of the need for exogenous insulin only if the disease process could be halted while the patient still possessed a sufficient number of beta cells to provide adequate amounts of endogenous insulin. Therefore, any form of therapy would

be more effective if persons at risk could be identified while they were yet without overt symptoms of IDDM and before the patients require exogenous insulin. About 90% of new cases of IDDM occur outside of families with known cases. Therefore, assays suitable for mass screening are urgently needed to detect the subclinical disease process at a stage before it is irreversible.

Fortunately, there are a variety of animal models for IDDM, including BB rats and NOD mice (for example, see Rossini et al., Ann. Rev. Immunol., 3:289-320, 1985). Many of the animals develop autoimmune IDDM spontaneously, and demonstrate many of the features of IDDM in humans.

Heat shock proteins (hsp's) are a family of proteins produced by cells exposed to elevated temperatures or other stresses. The hsp's include proteins of various molecular weights, including 20KD, 65-68KD, 70 KD, 90 KD, 110 KD, and others. The heat shock proteins are ubiquitous throughout nature; they are produced by bacteria, yeasts, plants, insects, and higher animals, including humans. The hsp protein molecules are highly conserved and show remarkable homology between all of these diverse creatures. Because of their extreme conservation over evolutionary time, heat shock proteins are thought to perform vital functions. They usually exhibit increased synthesis following exposure of cells to stressful stimuli including heat,

certain metals, drugs, or amino acid analogues. Nevertheless, the special functions of these proteins so far are obscure.

For example, patients with systemic lupus erythematosus (SLE) were observed to have antibodies to a 90 KD heat shock protein (Minota et al., J. Clin. Invest., 81:106-109, 1988). The function of these antibodies to hsp90 are not known.

Hsp65 was found to be involved in adjuvant arthritis in rats, cf. van Eden et al., Nature, 331:171-173, 1988. Adjuvant arthritis is an autoimmune arthritis triggered by immunizing certain strains of rats to Mycobacterium tuberculosis (MT) organisms. It was found that the disease could be transferred to immunologically naive, irradiated rats by a clone of T-lymphocytes reactive to a 9 amino acid peptide sequence (180-188) of the hsp65 of MT. Thus, adjuvant arthritis appeared to be an autoimmune disease produced by anti-hsp65 T-lymphocytes. The autoimmune attack against the joints was attributed to partial sequence homology between the 180-185 hsp65 peptide and a segment of the link protein of the cartilage proteoglycan (cf. Cohen, Scientific American, 256:52-60, 1988). It was also found that T-lymphocytes from the synovial fluids of patients with rheumatoid arthritis responded to the hsp65 of MT (cf. Res et al., Lancet, II:478-480, 1988).

Administration of hsp65 to rats before induction of adjuvant arthritis was found to prevent the later development of arthritis. Thus, the presence of an immune response to hsp65 was associated with arthritis in both rats and humans, and administration of hsp65 could lead to resistance to arthritis.

European patent application 262,710 discloses polypeptides useful for alleviation, treatment, and diagnosis of autoimmune arthritis and similar autoimmune diseases.

The complete primary structure, including nucleotide and deduced amino acid sequence of the human P1 protein has recently been published in Jindal, S. et al, "Primary Structure of a Human Mitochondrial Protein Homologous to the Bacterial and Plant Chaperonins and to the 65-Kilodalton Mycobacterial Antigen," Molecular and Cellular Biology, 9, 5, 2279-2283, 1989. This protein, disclosed as having a molecular weight of about 63 kDa, is the human heat shock protein referred to herein as the hHSP65 protein. The entire contents of this publication are hereby incorporated herein by reference. The structure of this proteir reproduced as Fig. 3 herein is intended to be identical to that disclosed in Jindal.

European patent application 261,648 discloses the use of activated T cells specific for an autoimmune disease

for the treatment of such disease. The T cells are preferably first pressure treated, subjected to a chemical cross-linking agent and/or subjected to a cytoskeletal disrupting agent in order to improve their immunogenicity. The entire treated cell or fraction thereof may be used as a vaccine against the autoimmune disease for which the T cell is specific.

In the known procedure for causing the arrest of autoimmune T cells, the subject is immunized with a sample of attenuated or avirulent T cells of the particular autoimmune specificity, or fragments or fractions thereof. The subject responds by activating regulatory T cells of at least two types: anti-ergotypic T cells that recognize T cell activation markers and anti-idiotypic T cells that appear to recognize the self-antigen receptors present on the pathogenic endogenous autoimmune T cells. T cell vaccination in experimental animals is effective in inducing permanent remission of established disease as well as in preventing disease. Howell et al, Science, 246:668-670, 1989, and Vandenbark et al, Nature, 341:541-544, 1989, disclose use of peptide sequences of a T cell receptor β chain for vaccination ofrats against experimental autoimmune encephalomyelitis, thereby supporting the conclusion that the autoimmune T cell receptor itself can supply a target epitope for regulator T cells.

While such use of T cells or fragments was known for autoimmune diseases in general, the particular antigen

specific for IDDM was not previously known and, thus, activated T cells for vaccination against IDDM were not obtainable prior to the present invention.

Summary of the Invention

It is an object of the present invention to provide methods for the early diagnosis of insulin dependent diabetes mellitus (IDDM).

It is a further object of the present invention to provide kits for use in the early diagnosis of IDDM.

It is another object of the present invention to provide methods for the prevention of IDDM.

It is yet another object of the present invention to provide methods for the treatment of IDDM in its incipient stages.

It is still a further object of the present invention to provide tolerogenic compositions for the prevention or treatment of IDDM.

It is yet a further object of the present invention to provide novel polypeptides which can be used for the prevention or treatment of IDDM.

It is still another object of the present invention to provide T cells or fragments useful for the prevention of IDDM or treatment of IDDM in its incipient stages.

It is a further object of the present invention to use the IDDM specific antigen of the present invention to isolate T cells specific thereto and then to characterize

the peptide sequence of the receptor region of such T cells and use such receptor peptides for the prevention or treatment of IDDM.

According to the discovery of the present invention, in the course of developing IDDM, animals express hsp65 molecules, or molecules which are cross-reactive therewith, which find their way into the blood and urine of the animals. They also express antibodies and T cells directed specifically to such molecules. Thus, the presence of hsp65 (or molecules which are cross-reactive therewith) or antibodies or T cells specific thereto in blood or urine, serves as an assay for the detection of the IDDM process before the destruction of beta cells is completed and the individual is doomed to life-long diabetes.

The presence or incipience of IDDM in a patient can be diagnosed by testing for the presence of hsp65 (or molecules which are cross-reactive therewith) or antibodies or T cells specific thereto.

The present invention also relates to means for performing such assays, as well as kits for performing such assays. The detection of incipient diabetes then permits a patient to begin measures aimed at terminating the autoimmune process. For example, the administration of hsp65, or an active epitope thereof or another molecule (antigen) which is immunologically cross-reactive therewith, is effective in inducing resistance to the autoimmune process involved in IDDM. Administration of T cells specific to such

antigens, in attenuated or avirulent form or after having been treated to improve their antigenicity, or fragments or active fractions thereof, will also serve to induce resistance to the autoimmune process involved in IDDM.

The present invention further relates to means for preventing or treating IDDM. It has been discovered that immunization to hsp65, or the active epitope thereof or another molecule (antigen) which is immunologically cross-reactive therewith, in an appropriate adjuvant can induce IDDM. However, vaccination with such an antigen, without an effective adjuvant, and preferably with a tolerogenic carrier, can produce a specific tolerance to the antigen. This effectively creates a resistance to the autoimmune process of IDDM. The same is true with respect to vaccination with T cells specific to such antigens, in attenuated or avirulent form or after having been treated to improve their antigenicity, or fragments or active fractions thereof. If the patient is shown to already be in the pre-clinical incipient stages of IDDM, injection with such an antigen or T cell (or fraction) can create a tolerance for this antigen and thus arrest the autoimmune process before significant, permanent damage is done.

Brief Description of the Drawings

The present invention will be better understood from the following brief description of the drawings and the subsequent detailed description of the preferred embodiments.

Figure 1 shows the amounts of hsp65, anti-hsp65, anti-insulin antibody, and anti-idiotypic antibody in the serum of NOD mice that did not develop IDDM.

Figure 2 is a graph showing that marked increases in hsp65 and anti-hsp65 precede the development of overt IDDM in NOD mice that did develop the disease. Anti-insulin and idiotypic (DM) antibodies preceded IDDM by a lesser extent.

Figure 3 shows the nucleotide and deduced amino acid sequences of the human P1 protein, which is an hHSP65. Numbers on the left refer to the nucleotide sequence relative to coordinate 1 at the beginning of the putative initiation codon. The amino acid sequence is numbered starting with 1 at the same point. The 5' extension of this reading frame is shown in one-letter code. The position of the internal EcoRI site (nt 712), which marks the beginning of the λ22a sequence, is indicated. The polyadenylation signal 15 nt from the A tail at the 3' end is underlined. The putative mitochondrial targeting sequence at the N-terminal end and a keratin-like amino acid sequence at the C-terminal end containing repeats of Gly-Gly-Met are boxed.

Positively charged amino acids in the leader sequence are identified (+).

Figure 4 is a graph showing the degree of spontaneous reactivity of NOD/Lt T cells to human hsp65, MT-hsp65 and MT-hsp70 as a function of age.

Figure 5 is a graph showing the T cell proliferative response to p277 and p278 as a function of the concentration of peptide.

Figure 6 is a bar graph showing the proliferation of C7 and C9 T cell clones, which are capable of transferring acute diabetes to young, prediabetic NOD/Lt mice, in response to p277, MT hsp65 and plasmid control.

Figure 7 shows the results of immunization against peptides p277 and p278 in resisting induced diabetes. The dots show the blood glucose level three weeks after immunization for each mouse in the test groups.

Detailed Description of Preferred Embodiments

The following examples show specific embodiments of the present invention and experiments relating to the present invention. These are intended as examples only and are presented in a non-limitative manner.

EXAMPLE 1: Production of the MT hsp65 Molecule

The hsp molecule of Mycobacterium tuberculosis was transfected into E. coli by standard procedures and purified as described by van Eden et al, Nature, 331:171-173, 1988.

Such genetically engineered E. coli cells will produce substantial quantities of MT hsp65. Because of the close homology between hsp's of various sources, hsp65 of mammalian or human origin is also effective when produced by genetic engineering or isolation from cells.

EXAMPLE 2: Production of Antibodies to MT hsp65

Rabbits of a standard laboratory strain (New Zealand White) were inoculated subcutaneously in the back with 100 micrograms of MT hsp65 produced in accordance with Example 1, in 0.5 ml saline emulsified in 0.5 ml mineral oil (incomplete adjuvant). One month later the rabbits were boosted with 100 micrograms of MT hsp65 in 1.0 ml saline, and two weeks later the rabbits were bled and the serum collected. The rabbits were boosted in a similar manner after two months and bled again. The sera antibodies were used to detect hsp65 in the blood and urine of test animals and humans.

EXAMPLE 3: Assay of hsp65

A standard solid phase radioimmunoassay is used to detect the presence of hsp65 molecule. Flexible PVC microtiter plates are coated with 100 µl test serum or urine for 18 hours at 4°C and washed with phosphate buffered saline (PBS). Control rabbit serum or anti-hsp65 serum (produced in accordance with Example 2) is then diluted 1:100 in PBS + 0.1% bovine serum albumin (BSA), and 50 µl is added to each

Table 1

Serum Assay of Impending IDDM in NOD Mice

Mouse	Day of IDDM onset	Days Positive Test Preceded IDDM Onset: hsp65	anti-hsp65	anti-insulin	anti-idiotype
1	none	0	0	0	0
2	none	0	0	0	0
3	none	0	0	0	0
4	none	0	0	0	0
5	185	90	45	45	30
6	185	100	50	45	20
7	170	90	60	60	30
8	170	100	50	30	20
9	145	60	60	25	15
10	145	70	30	15	15
11	145	60	40	20	20
12	130	50	20	0	0
13	115	55	55	20	20
14	115	50	30	30	20
Mean	150.5	72.5	44	29	19
SE	8.28	6.47	4.33	5.47	2.67
Median	145	65	47.5	27.5	20

The mean age of IDDM onset was 150.5 days in the mice developing disease. The mean hsp65 serum test was positive 72.5 days before IDDM and the mean anti-hsp65 test was positive 44 days before IDDM. The anti-insulin and

anti-idiotypic antibody tests were positive only 29 and 19 days before IDDM on the average. The tests were not significantly positive in mice escaping IDDM. Therefore, hsp65 and anti-hsp65 are relatively early indicators of eventual development of IDDM.

Urine was tested for the presence of hsp65 in the NOD mice at about 100 days of age. Table 2 shows that the urine of the mice tested positive in those mice that did develop IDDM.

Table 2

Urine Assay of Impending IDDM in NOD Mice

IDDM	Urines positive for hsp65
Yes	10/10
No	0/4

BB Rats

Table 3 shows that BB rats that did not develop IDDM did not manifest hsp65 or anti-hsp65 in the serum or urine. Rats that did develop IDDM (on days 90-100) were positive when tested 10 to 20 days before the outbreak of IDDM. The assays were conducted as disclosed in Examples 3 and 4.

Table 3

Assays of hsp65 and Anti-hsp65 Associated with Development of IDDM in BB Rats

Development of IDDM	Serum		Urine	
	hsp65	anti-hsp65	hsp65	anti-hsp65
Yes	10/10	5/5	4/5	3/5
No	0/5	0/5	0/5	0/5

Human IDDM Patients

Sera were available from five patients at various times before they developed IDDM. The sera were obtained from these persons 1/2 to 2 years before the onset of IDDM because they were first degree relatives of known IDDM patients and were thought to be at risk of developing IDDM themselves.

In addition to those persons, sera and urines of four newly diagnosed IDDM patients were studied for hsp65. Control sera and urines were obtained from 10 patients with active multiple sclerosis and 35 children seen at a general hospital for a variety of problems not related to IDDM. The results are shown in Table 4. The assays were conducted in accordance with the procedures of Examples 3 and 4.

Table 3

Assays of hsp65 and Anti-hsp65 Associated with Development of IDDM in BB Rats

Development of IDDM	Serum		Urine	
	hsp65	anti-hsp65	hsp65	anti-hsp65
Yes	10/10	5/5	4/5	3/5
No	0/5	0/5	0/5	0/5

Human IDDM Patients

Sera were available from five patients at various times before they developed IDDM. The sera were obtained from these persons 1/2 to 2 years before the onset of IDDM because they were first degree relatives of known IDDM patients and were thought to be at risk of developing IDDM themselves.

In addition to those persons, sera and urines of four newly diagnosed IDDM patients were studied for hsp65. Control sera and urines were obtained from 10 patients with active multiple sclerosis and 35 children seen at a general hospital for a variety of problems not related to IDDM. The results are shown in Table 4. The assays were conducted in accordance with the procedures of Examples 3 and 4.

Table 4

hHSP65 and anti-hHSP65 in human IDDM patients

Humans	Serum		Urine	
	hHSP65	anti-hHSP65	hHSP65	anti-hHSP65
Pre-IDDM	4/5	4/5	N.D.	N.D.
New IDDM	2/4	2/4	2/4	2/4
Multiple Sclerosis	0/10	0/10	N.D.	N.D.
Hospitalized Children (no IDDM)	0/35	0/35	N.D.	N.D.
Healthy adults	0/10	0/10	0/10	0/10

It can be seen from the above table that four out of five of the pre-IDDM patents and two out of four of the IDDM patients were positive in the hHSP65 and anti-hHSP65 assays. None of the controls was positive. Thus, anti-hsp65 raised in rabbits against hsp65 of MT can detect hHSP65 in human serum and urine in association with the development of IDDM. Moreover, hsp65 of MT could detect human antibodies. As discussed above, antibodies made to hsp65 of human or other origin can also be used in these assays, as well as hsp65 obtained from human or other sources. This is possible because of the high degree of conservation of hsp's throughout biological evolution.

That all of the pre-IDDM and new IDDM patients were not positive is explained by the fact that the concentrations of hHSP65 and anti-hHSP65 tend to decrease at or around the actual time of IDDM onset, as shown in Figure 2.

Thus, the negative patients may have lost their positivity when they were tested close to the onset of IDDM.

From the above, it is apparent that human patients will be positive for hHSP65 (or a molecule cross-reactive therewith) or anti-hHSP65 at some time early before the onset of IDDM. Assays for hHSP65 or anti-hHSP65 are therefore useful in screening populations for those that may be in the process of developing IDDM.

The hHSP65 appearing in the blood or urine of individuals developing IDDM could come from several sources. The sources may be hHSP65 expressed normally by healthy beta cells and released when the beta cells undergo viral infection or toxic insult as a prelude to immunological destruction, or it may be released from the beta cells by the stress of immunological destruction. The hHSP65 might also be expressed by the cells of the immune system during their prolonged activity against the beta cells. Although the sources of hHSP65 in the system are not at this time conclusively known, it has been determined that once the hHSP65 is released, the individual is stimulated to make antibodies to the hHSP65 molecule.

Antibodies to an undefined molecule of 64,000 molecular weight have been described in some newly diagnosed IDDM patients by Baekeskov et al. in Nature, 298: 167-168, 1982. However, it is not known whether the 64 KD antigen is an hsp. Moreover, the 64 KD antigen is not known to appear

EXAMPLE 10: HSP65 Can Induce Resistance to Induction of IDDM

It is well established that antigen administered without an effective adjuvant, or with a tolerogenic carrier, can induce immunological non-responsiveness, i.e., specific tolerance to the antigen. Therefore, mice that had been injected with hsp65 in PBS were tested to determine if these mice had acquired resistance to IDDM induced by hsp65 in oil. One month after receiving hsp65 in PBS, C57BL/Ksj mice were challenged with hsp65 in oil, and none of these mice developed IDDM as measured by blood glucose greater than 250 mg% three weeks later. In contrast, 8 of 10 control mice that had not received hsp65 in PBS developed IDDM after receiving hsp65 in oil.

In another experiment, hsp65 was given to 30 day old female NOD mice in PBS, intraperitoneally, 15 days before challenge with 50 μg hsp65 in oil to induce IDDM. The presence of IDDM was measured by blood glucose concentration of greater than 200 mg% 35 days after challenge. The presence of IDDM was again measured when the mice were 5 months of age. At this age it is known that 50% of all untreated female NOD mice have detectable IDDM. The results are shown in Table 8.

TABLE 8

Use of hsp65 to Vaccinate against IDDM

hsp65 in PBS (μg)	Incidence of IDDM: 35 days after challenge	Incidence of IDDM: 5 months old
0	7/8	
1	0/8	0/8
5	0/8	0/8
50	0/8	0/8

Thus, it can be seen that hsp65 can be used to induce tolerance to a diabetogenic immune process. Not only is this tolerance effective with respect to an immunogenic attack of hsp65, but it remains effective as a treatment against the natural development of spontaneous IDDM in NOD mice.

EXAMPLE 11: Treatment of Incipient IDDM Using hHSP65

As shown in Example 10, hsp65 can be used to induce resistance to the autoimmune process of IDDM. This appears to be caused by a mechanism of immunological tolerance to the hHSP65 of the beta cells through exposure to exogenous hsp65. Thus, hsp65 can be useful in treating IDDM before the disease becomes clinically evident and the autoimmune process can be arrested before significant, permanent damage is done. The results of the experiment summarized in Table 8 to the effect that the natural development of spontaneous IDDM in NOD mice can be arrested is significant

evidence that hsp65, and particularly hHSP65, can be used therapeutically. The autoimmune process begins very early in NOD mice. At the age of one month insulitis can already be detected. IDDM becomes clinically evident at 5 months in 50% of the female mice of this strain. Administration of hsp65 in 30 day old mice stops this natural development. This establishes that treatment can be effective even after autoimmunity to the islets has already begun.

EXAMPLE 12: T cell Response to hHSP65 is Associated with Developing Diabetes

The human hsp65 gene shown in Fig. 3 was cloned for expression in a conventional manner and substantially pure recombinant human hsp65 was obtained therefrom.

The present experiment establishes that mice spontaneously destroying their beta cells manifest T cell reactivity to recombinant human hsp65 Spleen cell suspensions obtained from groups of five to seven female NOD/Lt mice of various ages were assayed for T cell proliferation, essentially as described for T cell responses to thyroglobulin (Maron, R. et al., J. Immunol., 131, 2316-2322 (1983)). Briefly, the cells at 1 x 10^6 cells per ml were incubated in triplicate for 72 hours in 0.2 ml of culture medium in microtiter wells in the presence or absence of the following antigens at 5μg/ml: human hsp65, MT hsp65, or MT hsp70. Proliferation was measured by the incorporation of [^{3}H] thymidine into DNA during the final 12 hours of incubation. The results are shown as the Δ cpm:

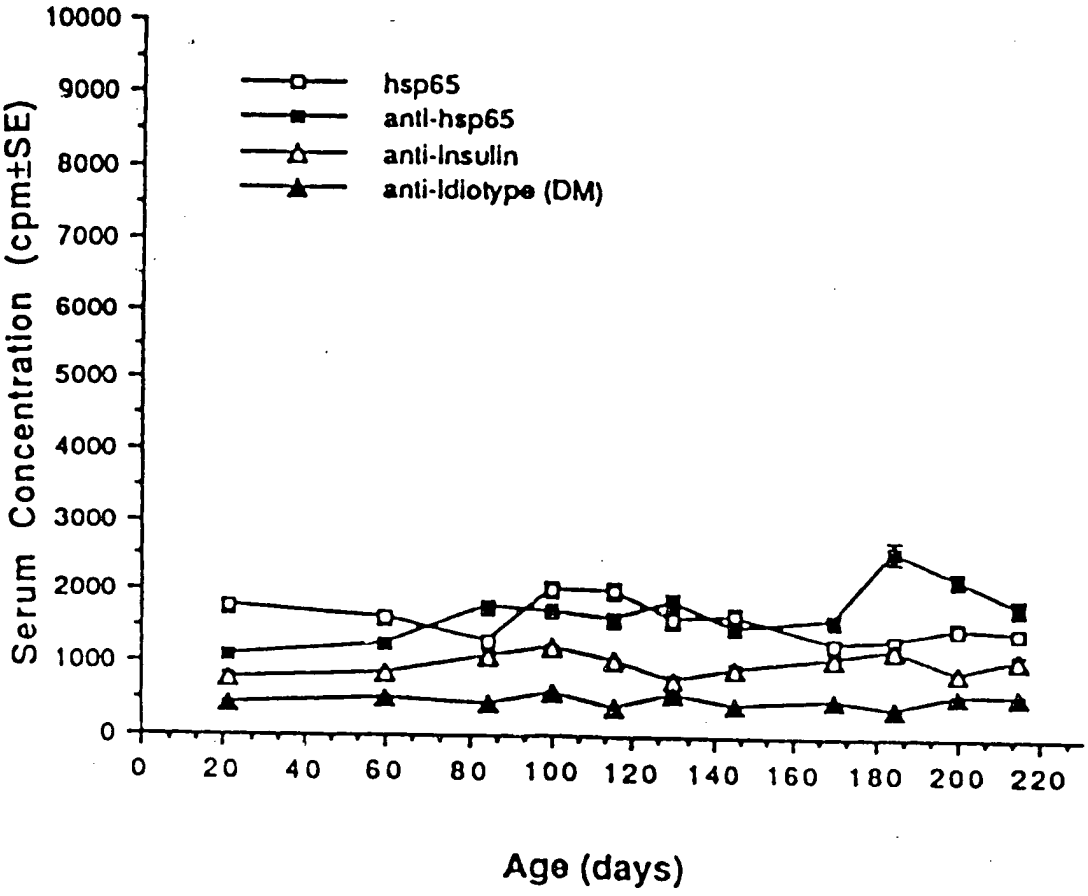
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Serum Concentration (cpm±SE)
hsp65
anti-hsp65
anti-Insulin
anti-Idiotype (DM)
0
20
40
60
80
100
120
140
160
180
200
220
Age (days)

FIG.1

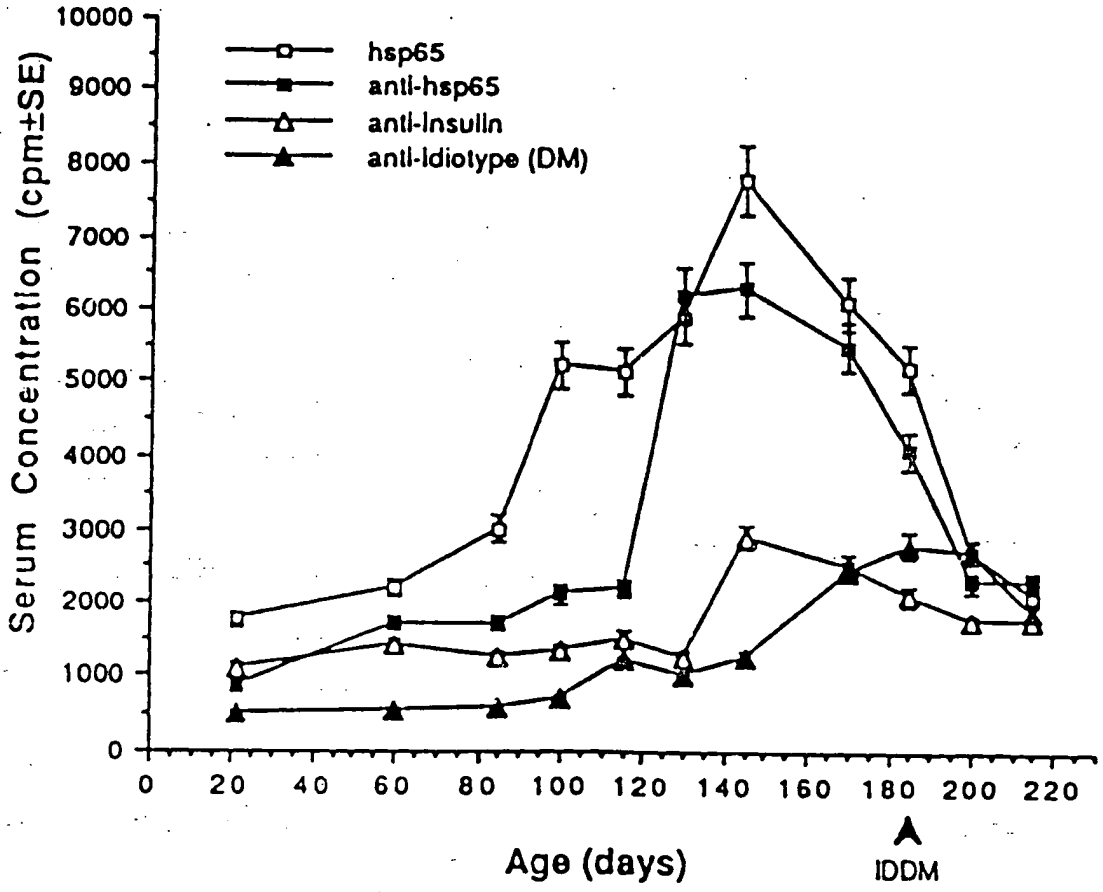
hsp65
anti-hsp65
anti-Insulin
anti-Idiotype (DM)
Serum Concentration (cpm±SE)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0 20 40 60 80 100 120 140 160 180 200 220
Age (days)
IDDM

FIG. 2

WHAT IS CLAIMED IS:

1. A method for diagnosing the presence or incipience of IDDM in a patient comprising testing the blood or urine of said patient for the presence of a protein which is immunologically reactive with antibodies raised against hHSP65 or for the presence of antibodies or T cells which are immunologically reactive with hHSP65.

2. A method in accordance with claim 1, wherein the blood is tested for the presence of said protein antibodies or T cells.

3. A method in accordance with claim 1, wherein the urine is tested for the presence of said protein or said antibodies.

4. A method in accordance with claim 1, wherein said testing step comprises testing the blood serum or urine of said patient for the presence of a protein which is immunologically reactive with antibodies raised against hHSP65.

5. A method in accordance with claim 1, wherein said testing step comprises testing the blood serum or urine of said patient for the presence of antibodies which are immunologically reactive with hHSP65.

6. A method in accordance with claim 1, wherein said testing step comprises testing the blood of said patient for the presence of T cells which are immunologically reactive with hHSP65.

7. A method in accordance with claim 4, wherein said protein is one which is immunologically reactive with antibodies raised against the p277 sequence of hHSP65.

8. A method in accordance with claim 5, wherein said antibodies are those which are immunologically reactive with the p277 sequence of hHSP65.

9. A method in accordance with claim 4, wherein said testing step comprises a radioimmunoassay.

10. A method in accordance with claim 4, wherein said testing step comprises an ELISA test.

11. A method in accordance with claim 5, wherein said testing step comprises injecting hHSP65 subcutaneously into a patient and observing the occurrence of a detectable skin reaction.

12. A kit for diagnosing for the presence of IDDM comprising hHSP65 idiotypic antibodies and a tagged antibody capable of recognizing the nonvariable region of hHSP65 antibody or anti-hHSP65 idiotypic antibody to be detected, and a reagent capable of providing a detectable signal for the presence of hHSP65 or antibodies thereto.

13. A kit in accordance with claim 12, wherein the hHSP65 idiotypic antibodies are immobilized on a solid phase, and the tagged antibody is Fab.

14. A kit in accordance with claim 12, wherein the tag is selected from the group consisting of radioisotopes, enzymes, chromophores, and fluorophores.

15. A method for the prevention or treatment of insulin dependant diabetes mellitus (IDDM), comprising:

prior to development of clinical IDDM, administering a substance which immunologically reacts with polyclonal antibodies raised against hHSP65 or which raises antibodies which immunologically react with hHSP65, in a manner which induces immunological tolerance to hHSP65.

16. A method in accordance with claim 15, for the treatment of IDDM, wherein the patient being treated is one who has been assayed positive for incipient IDDM but has not yet manifested clinical symptoms.

17. A method in accordance with claim 15, for the treatment of IDDM, wherein the patient has a detectable protein which is immunologically reactive with antibodies raised against hHSP65 or antibodies which are immunologically reactive with hHSP65 in the blood or urine but has not yet manifested clinical symptoms of IDDM developed.

18. A method in accordance with claim 15, wherein said substance being administered is in the form of a pharmaceutical composition of said substance in a pharmaceutically acceptable tolerogenic carrier.

19. A method in accordance with claim 15, wherein said substance comprises hsp65 or a salt, functional derivative, precursor or active fraction thereof.

20. A method in accordance with claim 15, wherein said substance comprises hHSP65 or a salt, functional derivative, precursor or active fraction thereof.

21. A method in accordance with claim 15, wherein said substance comprises hsp65.

22. A method in accordance with claim 21, wherein said hsp65 is hHSP65.

23. A method in accordance with claim 15, wherein said substance comprises p277 or a salt, functional derivative, precursor or active fraction thereof.

24. A preparation for preventing or treating insulin dependant diabetes mellitus (IDDM), comprising: (a) T cells which have developed specificity for a protein which is immunologically reactive with antibodies raised against hHSP65, which cells have been activated either by incubating in the presence of said protein or by incubating with a mitogen capable of inducing an immune response by the T cells; (b) said T cells which have been attenuated; (c) said T cells which have been subjected to pressure treatment by means of hydrostatic pressure, treatment with chemical cross-linking agent and/or treatment which a cytoskeletal cross-linking agent; (d) fragments of or surface proteins shed from (a), (b) or (c); or (e) a peptide consisting essentially of the variable region of the receptor of (a) specific for said protein, or a salt, functional derivative, precursor or active fraction thereof.

25. A pharmaceutical composition for the prevention or treatment of insulin dependent diabetes mellitus, comprising a pharmaceutically acceptable tolerogenic carrier and, as active principle, an effective amount of a substance which immunologically reacts with polyclonal antibodies raised against hHSP65 or which raises antibodies which immunologically react with hHSP65.

26. A pharmaceutical composition in accordance with claim 25, wherein said active principle is hsp65.

27. A pharmaceutical composition in accordance with claim 25, wherein said active principle is hsp65 or a salt, functional derivative, precursor or active fraction thereof.

28. A pharmaceutical composition in accordance with claim 25, wherein said active principle is hHSP65 or a salt, functional derivative, precursor or active fraction thereof.

29. A pharmaceutical composition in accordance with claim 28, wherein said active principle is hHSP65.

30. A pharmaceutical composition in accordance with claim 25, wherein said active principle is p27% or a salt, functional derivative, precursor or active fraction thereof.

31. A method for the prevention or treatment of insulin dependant diabetes mellitus (IDDM), comprising:

prior to development of clinical IDDM, administering a preparation in accordance with claim 24 in a manner which induces immunological tolerance to hHSP65.

32. A substantially pure polypeptide which includes the sequence Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-Cys-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Thr-Pro-Ala-Asn-Glu-Asp, or any modification thereof which is immunologically cross-reactive therewith, but which is not an entire heat shock protein.

33. A polypeptide in accordance with claim 32, wherein said polypeptide includes the sequence Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-Cys-Ile-Pro-Ala-Leu-Asp-Ser-Leu-Thr-Pro-Ala-Asn-Glu-Asp, or any modification thereof which retains at least 40% sequence homology therewith.

34. A polypeptide in accordance with claim 32 and having approximately 24 amino acids.